Safety and immunogenicity of Pfs25H-EPA/Alhydrogel, a transmission-blocking vaccine against *Plasmodium falciparum*: a randomised, double-blind, comparator-controlled, dose-escalation study in healthy Malian adults

Issaka Sagara, Sara A Healy*, Mahamadoun H Assadou, Erin E Gabriel, Mamady Kone, Kourane Sissoko, Intimbeye Tembine, Merepen A Guindo, M'Bouye Doucoure, Karamoko Niaré, Amagana Dolo, Kelly M Rausch, David L Narum, David L Jones, Nicholas J MacDonald, Daming Zhu, Rathy Mohan, Olga Muratova, Ibrahima Baber, Mamadou B Coulibaly, Michael P Fay, Charles Anderson, Yimin Wu, Sekou F Traore, Ogobara K Doumbo, Patrick E Duffy*

Summary

Background Pfs25H-EPA is a protein-protein conjugate transmission-blocking vaccine against *Plasmodium falciparum* that is safe and induces functional antibodies in malaria-naive individuals. In this field trial, we assessed Pfs25H-EPA/Alhydrogel for safety and functional immunogenicity in Malian adults.

Methods This double-blind, randomised, comparator-controlled, dose-escalation trial in Bancoumana, Mali, was done in two staggered phases, an initial pilot safety assessment and a subsequent main phase. Healthy village residents aged 18–45 years were eligible if they had normal laboratory results (including HIV, hepatitis B, hepatitis C tests) and had not received a previous malaria vaccine or recent immunosuppressive drugs, vaccines, or blood products. Participants in the pilot safety cohort and the main cohort were assigned (1:1) by block randomisation to a study vaccine group. Participants in the pilot safety cohort received two doses of Pfs25H-EPA/Alhydrogel 16 µg or Euvax B (comparator vaccine), and participants in the main cohort received Pfs25H-EPA/Alhydrogel 47 µg or comparator vaccine (Euvax B for the first, second, and third vaccinations and Menactra for the fourth vaccination). Participants and investigators were masked to group assignment, and randomisation codes in sealed envelopes held by a site pharmacist. Vials with study drug for injection were covered by opaque tape and labelled with a study identification number. Group assignments were unmasked at final study visit. The primary outcomes were safety and tolerability for all vaccinees. The secondary outcome measure was immunogenicity 14 days after vaccination in the per-protocol population, as confirmed by the presence of antibodies against Pfs25H measured by ELISA IgG and antibody functionality assessed by standard membrane feeding assays and by direct skin feeding assays. This trial is registered with ClinicalTrials.gov, number NCT01867463.

Findings Between May 15, and Jun 16, 2013, 230 individuals were screened for eligibility. 20 individuals were enrolled in the pilot safety cohort; ten participants were assigned to receive Pfs25H-EPA/Alhydrogel 16 µg, and ten participants were assigned to receive comparator vaccine. 100 individuals were enrolled in the main cohort; 50 participants were assigned to receive Pfs25H-EPA/Alhydrogel 47 µg, and 50 participants were assigned to receive comparator vaccine. Compared with comparator vaccinees, Pfs25H vaccinees had more solicited adverse events (137 events *vs* 86 events; p=0·022) and treatment-related adverse events (191 events *vs* 126 events, p=0·034), but the number of other adverse events did not differ between study vaccine groups (792 *vs* 683). Pfs25H antibody titres increased with each dose, with a peak geometric mean of 422·3 ELISA units (95% CI 290–615) after the fourth dose, but decreased relatively rapidly thereafter, with a half-life of 42 days for anti-Pfs25H and 59 days for anti-EPA (median ratio of titres at day 600 to peak, 0·19 for anti-Pfs25H *vs* 0·29 for anti-EPA; p=0·009). Serum transmission-reducing activity was greater for Pfs25H than for comparator vaccine after the fourth vaccine dose (p<0·001) but not after the third dose (p=0·09). Repeated direct skin feeds were well tolerated, but the number of participants who infected at least one mosquito did not differ between Pfs25H and comparator vaccinees after the fourth dose (p=1, conditional exact).

Interpretation Pfs25H-EPA/Alhydrogel was well tolerated and induced significant serum activity by standard membrane feeding assays but transmission blocking activity was not confirmed by weekly direct skin feed. This activity required four doses, and titres decreased rapidly after the fourth dose. Alternative antigens or combinations should be assessed to improve activity.

Funding Division of Intramural Research, National Institute of Allergy and Infectious Diseases.

Lancet Infect Dis 2018; 18: 969–82

Published **Online** July 27, 2018 http://dx.doi.org/10.1016/S1473-3099(18)30344-X

See **Comment** page 927

*Contributed equally

Malaria Research and Training Center, Mali-National Institute of Allergy and Infectious Diseases International Center for Excellence in Research, University of Science, Techniques and Technologies of Bamako, Mali (I Sagara MD, M H Assadou MD, M Kone MD, K Sissoko MD, I Tembine MD, M A Guindo PharmD, M Doucoure BS, K Niaré PharmD, A Dolo PharmD, I Baber PhD, M B Coulibaly PhD, Prof S F Traore PhD, Prof O K Doumbo MD); **Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, USA** (S A Healy MD, K Rausch MS, D L Narum PhD, D L Jones PhD, N J MacDonald PhD, D Zhu MS, R Mohan MS, C Anderson PhD, O Muratova MS, Y Wu PhD, P E Duffy MD); **Biostatistics Research Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA** (M P Fay PhD); **and Department of Medical Epidemiology and Biostatistics, Karolinska Institute, Stockholm, Sweden** (E Gabriel PhD)

Correspondence to:
Dr Patrick E Duffy, Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892-8180, USA
patrick.duffy@nih.gov

Research in context

Evidence before this study

We searched PubMed, the Cochrane Library, Google Scholar, Scopus, and Web of Science on Feb 20, 2018, for articles in English about randomised controlled trials of malaria vaccines in adults since Jan 1, 1980. We searched using the terms ("malaria vaccines" [MeSH Terms] OR "malaria" [All Fields] AND "vaccines" [All Fields]) OR "malaria vaccines" [All Fields] OR ("malaria" [All Fields] AND "vaccine" [All Fields]) OR ("malaria vaccine" [All Fields]) AND (Transmission blocking vaccines [All Fields]) AND ("adults" [MeSH Terms] OR "adults" [All Fields]). For the Cochrane Library and other data sources, we used the key search terms "Transmission blocking vaccine", "malaria vaccines", "adults", AND "clinical trials". We did not identify any previous studies of the safety or immunogenicity of a malaria transmission-blocking vaccine in an endemic area. Previous trials of transmission-blocking vaccines based on Pfs25 or the *Plasmodium vivax* orthologue Pvs25 in malaria-naive volunteers in the USA did not meet safety or immunogenicity criteria to advance to field trials. Efforts are ongoing to develop other transmission-blocking vaccines based on parasite antigens, such as Pfs230 and Pfs48/45, or mosquito antigens, such as AnAPN1, but there are no reports of results from human trials with these candidates.

Added value of this study

This is the first report of the safety and immunogenicity of a malaria transmission-blocking vaccine in a naturally exposed population. Pfs25H-EPA in Alhydrogel delivered by intramuscular injection was well tolerated and safe, and induced significant functional activity that blocked parasite transmission in a laboratory assay; however, this activity was only seen at peak titres after four vaccine doses, and antibody titres rapidly waned.

Implications of all the available evidence

The findings from our study in Mali are the first to show that functional transmission-blocking antibodies can be induced in the target population, and they lay the foundation for further development of this class of vaccine that can contribute to malaria elimination and eradication. Pfs25H-EPA in Alhydrogel is safe and well tolerated, but it is necessary to improve vaccine activity and durability, possibly with alternative antigens or antigen combinations.

Introduction

Plasmodium falciparum is the deadliest of human malaria parasite species. Globally, although the number of deaths from malaria has decreased by more than a third in the past decade as control measures have been scaled up, 730 000 people died from malaria in 2015.[1] Policy makers and researchers have increasingly focused on malaria eradication as the only sustainable solution. Eradication will rely on effective interventions to interrupt transmission,[2] and vaccines have been key for smallpox and polio eradication programmes.

Malaria transmission-blocking vaccines are based on the insight that human antibodies can attack parasites in the mosquito.[3,4] Whole-gamete vaccination of animals induced antibodies against mosquito sexual-stage but not blood-stage parasites;[3] transmission-blocking vaccines can therefore block onward transmission without directly reducing host infection. Monoclonal antibodies generated by whole-organism vaccination have been used to identify candidate antigens for transmission-blocking vaccines. These include gamete surface proteins P230 and P48/45, which are first expressed by gametocytes in human blood,[5] and zygote surface proteins P25 and P28, which are expressed after fertilisation in the mosquito.[6,7] These antigens are multidomain cysteine-rich proteins and are generally difficult to produce as properly folded recombinant protein. *P falciparum* P25 (Pfs25) antigen was the first recombinant protein to be expressed successfully[8] and is the leading candidate for transmission-blocking vaccines.

See Online for appendix

Vaccines based on Pfs25 or its *Plasmodium vivax* orthologue Pvs25 have not advanced clinically because of poor immunogenicity[9,10] or excessive reactogenicity that is thought to be related to adjuvant formulations.[11] We previously reported that conjugation to immunogenic carriers enhances antibody titre of a transmission-blocking vaccine and its duration in animals.[12,13] In a US trial,[14] recombinant pichia-expressed, His-tagged Pfs25 conjugated to an *Escherichia coli*-expressed recombinant *Pseudomonas aeruginosa* ExoProtein A (EPA) and formulated in Alhydrogel (Pfs25H-EPA/Alhydrogel) induced functional antiserum in malaria-naive volunteers and reduced *P falciparum* transmission to mosquitoes in a laboratory assay. Antibody titre and avidity increased progressively from second to fourth dose, and titre correlated with serum functional activity after final dose.[14]

Here we report the first trial of a transmission-blocking vaccine against malaria in an adult malaria-exposed target population. We tested the safety of Pfs25H-EPA/Alhydrogel and whether functional antibody induced in Malian adults who receive Pfs25H-EPA/Alhydrogel reduced parasite transmission to *Anopheles stephensi* mosquitoes in laboratory assays and to *Anopheles coluzzii* in direct skin feed assays.

Methods

Study design and participants

This double-blind, randomised, comparator-controlled trial was done in and around Bancoumana, a rural village 60 km southwest of Bamako, Mali, with about 10 000 inhabitants. Malaria is hyperendemic in this region, with highly seasonal transmission from June to December (appendix p 16). This trial was done in accordance with Good Clinical Practice guidelines and institutional

procedures and guidelines. Each village provided community permission, and all participants provided written informed consent. The study was approved by the Mali ethics review board (Faculté de Médecine de Pharmacie et d'OdontoStomatologie, Bamako), the US National Institute of Allergy and Infectious Diseases (NIAID, National Institutes of Health, Bethesda, MD, USA) institutional review board, and the Mali national regulatory authority. The in-vestigational new drug identifier was FDA IND 14781.

Healthy men or non-pregnant, non-breastfeeding women aged 18–45 years were eligible if they were available for the trial duration, were known village residents, and were willing to participate in mosquito direct skin feeding (DSF) assays. Women of child-bearing potential were required to use reliable contraception throughout the vaccination period. Individuals were excluded if laboratory test results were abnormal (including HIV, hepatitis B, hepatitis C tests) or if they had received a previous malaria vaccine or recent immunosuppressive drugs, vaccines, or blood products. A full list of inclusion and exclusion criteria is provided in the appendix (pp 6–7).

Randomisation and masking

For safety reasons, the trial progressed in two staggered phases, with an initial pilot safety cohort and a subsequent main cohort staggered as two vaccination groups (appendix pp 14–15). Participants were block-randomised (1:1) using R to a study vaccine group. Participants in the pilot safety cohort were to receive two doses of Pfs25H-EPA/Alhydrogel 16 µg or Euvax B (comparator vaccine), and participants in the main cohort were to receive Pfs25H-EPA/Alhydrogel 47 µg or Euvax B (comparator vaccine for the first, second, and third vaccinations) and Menactra (comparator vaccine for the fourth vaccination; appendix p 12). Participants and investigators were masked to group assignments, and randomisation codes in sealed envelopes held by a site pharmacist. Vials with study drug for injection were covered by opaque tape and labelled with a study identification number. Group assignments were unmasked at the final study visit, which was scheduled 4 months after the second vaccination in the pilot safety cohort (to promote completion of hepatitis B vaccination series) and 6 months after the fourth vaccination in the main cohort. After unmasking, all Pfs25H vaccinees were offered Euvax B and Menactra.

Procedures

The Pfs25H-EPA/Alhydrogel vaccine contained *Pichia pastoris*-expressed 6-His-tagged recombinant Pfs25 conjugated to EPA (Walter Reed Army Institute of Research Pilot Bioproduction Facility, Silver Spring, MD, USA) and adjuvanted with Alhydrogel (Brenntag; Frederikssund, Denmark). Each vial contained 78 µg/mL conjugated Pfs25H, 93 µg/mL conjugated EPA, and 1600 µg/mL Alhydrogel in 0·8 mL volume. Participants in the pilot safety cohort received 0·2 mL injections of Pfs25H 16 µg at days 0 and 56, and participants in the main cohort received 0·6 mL injections of Pfs25H 47 µg at days 0, 56, 112, and 480. Licensed comparator vaccine Euvax B (1·0 mL recombinant hepatitis B vaccine; LG Life Sciences, Jeonbuk-do, South Korea) was given at days 0 and 56 in the pilot safety cohort and at days 0, 56, and 112 in the main cohort. Menactra (0·5 mL meningococcal polysaccharide vaccine for *Neisseria meningitidis* sero-groups A, C, Y, and W-135; Sanofi Pasteur, Swiftwater, PA, USA) was given at day 480 in the main cohort. Local paediatricians experienced in vaccine administration and clinical trial procedures completed vaccinations in deltoid muscles of alternating arms, and study clinicians did the follow-up and adverse event assessment. Participants were considered enrolled after their first vaccination.

To detect adverse events, participants were monitored for 30 min after the vaccination, on days 1, 3, 7, 14, and 28, and about monthly thereafter (until unmasking). Study clinical personnel were always available for unscheduled visits. Solicited local adverse events were recorded for 14 days, and systemic adverse events were recorded for 28 days after vaccinations (appendix p 16). Unsolicited adverse events including symptomatic malaria, serious adverse events, and new onset chronic illnesses, were recorded throughout the study. Serious adverse events included death, life-threatening events, inpatient hospital admissions, persistent or clinically significant incapacities, congenital anomalies, or medically important events. Protocol-specified laboratory tests, including complete blood count with differential, creatinine and alanine aminotransferase measurements, and urinalysis, were completed before and on days 3 and 14 after vaccination. Grading of adverse events was based on guidelines for vaccine clinical trials by the US Food and Drug Administration[15] and adapted to local normal reference ranges (appendix pp 17–18).

Blood smears were prepared before each vaccination, at least monthly after vaccination, or when clinically indicated. Starting 2 weeks after the third and fourth vaccinations, blood smears were prepared at every DSF visit. Symptomatic malaria was defined as asexual parasitaemia with axillary temperature of at least 37·5°C, clinical signs and symptoms of malaria, or both. Artemether plus lumefantrine was provided for symptomatic malaria, and asymptomatic parasitaemia was not treated, in accordance with Malian Government guidelines. Blood smears were examined by trained technicians using standard procedures.

Pfs25 and EPA antibody titres were measured by ELISA on the day of vaccination, 14 days after vaccination, and periodically after the third and fourth doses, using methods previously described.[14] Half-lives of both anti-Pfs25H and anti-EPA antibodies were calculated using the following equation, where N_0 is the starting

titre (day 494), N_t is the ending titre (day 600), t is time in days, and $t_{1/2}$ is half-life in days.

$$N_t = N_0 \times \frac{1}{2}^{t/t_{1/2}}$$

Antibody avidity was measured with modified ELISA using 6 M urea during washing. Avidity index was the ratio of optical density value, with urea over the optical density without urea in a Tris buffered saline solution with Tween. Functional activity was measured with the standard membrane feeding assay (SMFA) on serum samples.[14] Transmission-reducing activity was calculated with the following equation:

$$\text{Transmission-reducing activity} = \frac{\text{mean oocyst count}_{\text{control}} - \text{mean oocyst count}_{\text{test}}}{\text{mean oocyst count}_{\text{control}}} \times 100$$

Day 0 transmission-reducing activity was calculated with the following equation:

$$\text{Day 0 transmission-reducing activity} = \frac{\text{mean oocyst count}_{\text{Day 0 test}} - \text{mean oocyst count}_{\text{test}}}{\text{mean oocyst count}_{\text{Day 0 test}}} \times 100$$

Transmission-blocking activity was calculated with the following equation:

$$\text{Transmission-blocking activity} = \frac{\text{mean prevalence}_{\text{control}} - \text{mean prevalence}_{\text{test}}}{\text{mean prevalence}_{\text{control}}} \times 100$$

DSF was done weekly for 6 weeks for participants in the main cohort who had positive blood smear results (asexual or sexual), starting 14 days after the third vaccination (from Sept 29, to Nov 19, 2013), and for all participants in the main cohort starting 14 days after the fourth vaccination (from Sept 9, to Nov 29, 2014). Briefly, trained staff placed two mesh-covered cups with up to 30 prestarved, lab-adapted female *Anopheles coluzzii* mosquitoes on the participant's calf for 15–20 mins. Participants were then offered topical antihistamines or topical antipruritics (or both) and were followed actively for 24–48 h for any adverse events. Blood-fed mosquitoes were transported back to Bamako, stored in secure insectary, and dissected 1 week later for oocyst counts. Further details about the DSF procedure are provided in the appendix (pp 9–10).

Although the study protocol specified direct membrane feeding assays (DMFA) for measuring transmission-blocking activity as a secondary outcome, our pilot studies indicated that DSF was equally sensitive to DMFA but logistically simpler, so we used DSF as the only measure of functional activity in the field.

We assessed potential co-infections in the main cohort before the fourth vaccination, using previously published methods. Haemoglobinopathy was not exclusionary unless clinically relevant. Haemoglobin typing was completed retrospectively.

Outcomes

The primary objective was to assess safety, tolerability, and reactogenicity of repeated immunisation with increasing doses (16 µg or 47 µg) of Pfs25H-EPA/Alhydrogel. This was assessed by the occurrence and severity of local and systemic adverse events within 14 days (local) or 28 days (systemic) after each vaccination and the occurrence of serious adverse events. The secondary outcome measure was immunogenicity on day 14 after vaccination. Immunogenicity was confirmed by the presence of antibodies against Pfs25, as measured by ELISA IgG, and by antibody functionality, as assessed by SMFA and DSF assays.

Statistical analysis

Sample size calculations were based on the primary safety endpoint for both the pilot and main cohort but also took into account the secondary functional activity objectives for the main cohort (appendix pp 11–12).

All participants in the pilot safety study and the main cohort who received at least one dose of vaccine were included in safety analyses. Safety signals were investigated by the proportion of participants and the count reports overall and for a given adverse event of a specific grade and relationship to vaccination. We used Fisher's exact tests to compare proportions and Wilcoxon-Mann-Whitney tests to compare adverse event counts.

The secondary outcome measure was assessed in the per-protocol population. We compared immunogenicity between groups by the Wilcoxon-Mann-Whitney test at specific timepoints and by linear generalised estimating equations (GEE) and linear mixed effects models conditional on participant over all timepoints. We used the R packages gee,[16] lme4,[17] and lmerTest[18] for these repeated measures analyses, respectively. We compared seroconversion rates using conditional exact test for given timepoints (R package exact2x2). ELISA titres are described using geometric means, which were generated in Prism v7 (GraphPad Software, San Diego, CA).

Functional activity was assessed by SMFA and DSF assays. SMFA results were compared using Wilcoxon-Mann-Whitney for each timepoint separately, and their association with longitudinal ELISA values and EC_{50} were investigated using GEE. The DSF assay results were analysed using many different methods, including logistic and count mixed-effects models at the mosquito and DSF level (including random intercepts for DSF and participant), respectively. Logistic and count GEE models were also fit at mosquito and DSF levels. Both the GEE and mixed-effects models were run for all participants and for the subgroup of participants with detectable gametocytes

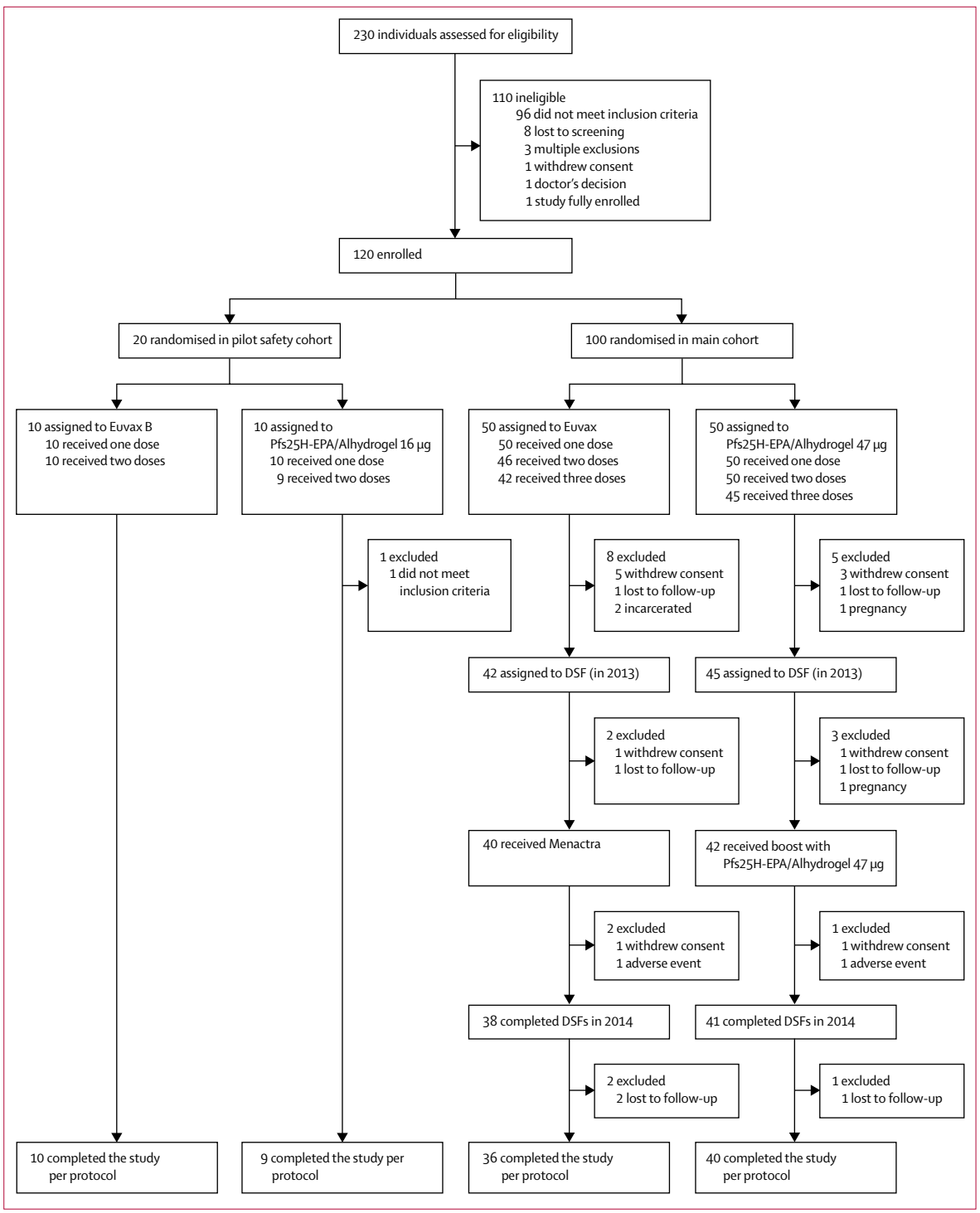


Figure 1: **Trial profile**
79 participants in the main cohort completed 461 DSFs. Study completion was defined as staying in the study until the end of the trial (study day 660).
DSF=direct skin feeding.

at the time of the DSF assay. The proportion of participants with at least one positive DSF assay result after the fourth vaccination was also compared between groups by conditional exact test. No adjustment for multiple comparisons was made. Further details are provided in the appendix (pp 12–13).

The study was monitored for safety by an independent data and safety monitoring board and a local medical

monitor. This trial is registered at ClinicalTrials.gov, number NCT01867463.

Role of the funding source

Scientists at National Institute of Allergy and Infectious Diseases but not officials were involved in study design, study management, data collection, data analysis, data interpretation, and writing of the report. IS and SAH had full access to all study data and had final responsibility for the decision to submit for publication.

Results

Between May 15, and Jun 16, 2013, 230 individuals were screened. 20 individuals were enrolled in the pilot safety cohort; ten participants were assigned to receive Pfs25H-EPA/Alhydrogel 16 µg and ten participants were assigned to receive the comparator vaccine (figure 1). First vaccinations in the pilot safety cohort were given in May, 2013, and the second vaccinations were given in June, 2013. Scheduled unmasking was done throughout November, 2013. Ten participants who received comparator vaccine and nine individuals who received Pfs25H 16 µg completed two vaccinations and were followed-up until the end of the study. One participant who received Pfs25H 16 µg did not meet eligibility criteria to continue after receipt of the first vaccination because of slightly increased creatinine concentration at baseline (grade 1 abnormality).

100 individuals were enrolled in the main cohort; 50 participants were assigned to receive Pfs25H-EPA/Alhydrogel 47 µg and 50 participants were assigned to receive comparator vaccine in two randomised sets, staggered by 1 week for safety reasons. All participants that entered randomisation received at least one vaccination and were eligible for safety analyses. In this main cohort, 42 comparator vaccinees received three doses and 40 comparator vaccinees received booster doses the following year. 45 Pfs25H vaccinees received three doses and 42 Pfs25H vaccinees received booster doses the following year. Of those who received booster doses, 38 comparator vaccinees and 41 Pfs25H vaccinees completed DSF assessments, whereas 36 comparator vaccinees and 40 Pfs25H vaccinees completed the end-of-study visit (figure 1).

Most participants were men from Bancoumana village with haemoglobin AA (table 1). The mean age was 34·4 years (SD 7·7), and the mean weight was 65·6 kg (SD 10·2).

Participants who received Pfs25H 16 µg or 47 µg had a good safety and tolerability profile, with most adverse events being grade 1 or grade 2 (appendix p 19–21). Total number of adverse events, local reactogenicity, laboratory result abnormalities (including neutropenia), or unsolicited adverse events did not differ significantly between participants in the Pfs25H and comparator vaccine groups, based on the number of unique individuals having at least one vaccination (table 2; appendix p 19–27). Pfs25H vaccinees (16 µg and 47 µg combined) had more solicited systemic adverse events (p=0·022; Fishers exact) and related adverse events (p=0·034; Fishers exact) than comparator vaccinees; the number of solicited systemic adverse events and related adverse events were also significantly different when comparing the count and rate (count per time at risk) per participant (table 2; appendix pp 19–25). These between-group differences did not increase with increasing number of doses.

In the pooled cohort of Pfs25H vaccinees (16 µg and 47 µg), the most common solicited adverse events included injection site-related events (42 [70%] of 60 Pfs25H vaccinees *vs* 35 [58%] of 60 comparator vaccinees) and headache (18 [30%] *vs* 13 [22%]); most adverse events were grade 1 or grade 2. One comparator vaccinee had

	Pilot safety cohort		Main cohort		Total (N=120)
	Pfs25H, 16 µg (n=10)	Comparator vaccine (n=10)	Pfs25H, 47 µg (n=50)	Comparator vaccine (n=50)	
Sex					
Male	7 (70%)	5 (50%)	34 (68%)	41 (82%)	87 (72·5%)
Female	3 (30%)	5 (50%)	16 (32%)	9 (18%)	33 (27·5%)
Age, years					
Mean (SD)	39·8 (4·7)	34·7 (7·9)	33·4 (8·1)	34·2 (7·4)	34·4 (7·7)
Range	32–44	18–44	18–44	19–44	18–44
Weight, kg					
Mean (SD)	65·2 (15·3)	66·0 (11·7)	64·6 (9·8)	66·7 (9·2)	65·6 (10·2)
Range	47–97	52–90	45–87	51–89	45–97
Village					
Bancoumana	10 (100%)	10 (100%)	18 (36%)	18 (36%)	56 (47%)
Samako	0	0	18 (36%)	14 (28%)	32 (27%)
Kolle	0	0	2 (4%)	3 (6%)	5 (4%)
Siranikoro	0	0	3 (6%)	2 (4%)	5 (4 %)
Djiguidala	0	0	6 (12%)	6 (12%)	12 (10%)
Gonsolo	0	0	2 (4%)	3 (6%)	5 (4%)
Missira	0	0	1 (2%)	4 (8%)	5 (4%)
Co-infections*					
Schistosoma haematobium	..	..	0	0	0
Helminth	..	..	2 (5%)	4 (11%)	6 (8%)
Protozoa	..	..	6 (15%)	6 (16%)	12 (15%)
Haemoglobinopathies†					
Hb AA	9 (100%)	5 (50%)	37 (74%)	35 (71%)	86 (73%)
Hb AS	0	2 (20%)	10 (20%)	9 (18%)	21 (18%)
Hb SC	0	0	0	2 (4%)	2 (2%)
Hb SC	0	0	0	0	0
Hb CC	0	1 (10%)	0	0	1 (1%)
Hb AC	0	2 (20%)	3 (6%)	3 (6%)	8 (7%)

Data are n (%), unless otherwise specified. *Co-infections were not measured at baseline; they were assessed before the fourth vaccination between August and September, 2014. 82 participants in the main cohort only (43 in the Pfs25H vaccine group; 39 in the comparator group) were evaluated for urinary schistosomiasis. 79 participants in the main cohort only (41 in the Pfs25H vaccine group; 38 participants in the comparator vaccine group) were assessed for stool parasites. One participant in the comparator group was positive for both helminth and protozoa and is counted once in each category. †Haemoglobin typing was completed for 19 participants in the pilot safety cohort (nine Pfs25H vaccinees; ten comparator vaccinees) and for 99 participants in the main cohort (50 Pfs25H vaccinees; 49 comparator vaccinees).

Table 1: **Characteristics of vaccinated participants**

grade 3 injection site pain (table 2; appendix p 19–21). Injection site pain was the most common injection site-related event (41 [68%] of 60 Pfs25H vaccinees *vs* 34 [58%] of 60 comparator vaccinees). The number of injection site-related events per participant was similar between groups and did not consistently increase with successive vaccine doses; however, by overall count and rate, there were more reported local reactogenicity events, including injection site pain, in the Pfs25H 47 µg vaccine group than in the comparator vaccine group (appendix p 22–23).

The most common unsolicited adverse events were symptomatic malaria, cold, and rhinitis. 26 grade 3 adverse events were reported (12 events in Pfs25H vaccinees *vs* 14 events in comparator vaccinees), all of which were deemed unrelated or unlikely to be related to the vaccine, except for one episode of injection site pain in a participant who received comparator vaccine. Three serious adverse events (one spontaneous abortion in the Pfs25H group; one snake bite and one trauma in the comparator vaccine group) were reported, all of which were deemed unrelated to the vaccine; two of these participants were excluded from further vaccination. No participants were removed from the study because of a related adverse event of any severity.

The study was stopped once because of several cases of grade 2 neutropenias, and the study was restarted after

	Pilot safety cohort				Main cohort				Total			
	Pfs25H, 16 µg		Comparator vaccine		Pfs25H, 47 µg		Comparator vaccine		Pfs25H		Comparator vaccine	
	Total number	Number of individuals (%)	Total number	Number of individuals (%)	Total number	Number of individuals (%)	Total number	Number of individuals (%)	Total number	Number of individuals (%)	Total number	Number of individuals (%)
Any adverse event												
Total	72	10 (100%)	89	10 (100%)	857*	50 (100%)	680*	49 (98%)	929	60 (100%)	769	59 (98%)
After first dose	23	9 (90%)	16	8 (80%)	135	45 (90%)	110	45 (90%)	158†	54 (90%)	126†	53 (88%)
After second dose	49	9 (100%)	73	10 (100%)	152	46 (92%)	121	39 (85%)	201	55 (93%)	194	49 (88%)
After third dose	N/A	N/A	N/A	N/A	367	45 (100%)	288	42 (100%)	367	45 (100%)	288	42 (100%)
After fourth dose	N/A	N/A	N/A	N/A	203	42 (100%)	161	40 (100%)	203	42 (100%)	161	40 (100%)
Related to vaccine	19	8 (80%)	10	5 (50%)	172*	48 (96%)	116*	42 (84%)	191*	56 (93%)‡	126*	47 (78%)‡
Solicited	6	4 (40%)	8	4 (40%)	131*	46 (92%)‡	78*	33 (66%)‡	137*	50 (83%)‡	86*	37 (62%)‡
Unsolicited	66	10 (100%)	81	10 (100%)	726	50 (100%)	602	49 (98%)	792	60 (100%)	683	59 (98%)
Total serious adverse events	0	0 (0%)	0	0 (0%)	1	1 (2%)	2	2 (4%)	1	1 (2%)	2	2 (3%)
Serious adverse event related to vaccine	0	0 (0%)	0	0 (0%)	0	0 (0%)	0	0 (0%)	0	0 (0%)	0	0 (0%)
Local reactogenicity												
Total	5	3 (30%)	7	4 (40%)	87*	39 (78%)	55*	31 (62%)	92*	42 (70%)	62*	35 (58%)
After first dose	3	2 (20%)	3	2 (20%)	23	21 (42%)	15	15 (30%)	26	23 (38%)	18	17 (28%)
After second dose	2	2 (22%)	4	3 (30%)	29†	26 (52%)‡	14†	13 (28%)‡	31†	28 (47%)	18†	16 (29%)
After third dose	N/A	N/A	N/A	N/A	17	17 (38%)	20	20 (48%)	17	17 (38%)	20	20 (48%)
After fourth dose	N/A	N/A	N/A	N/A	18†	18 (43%)‡	6†	6 (15%)‡	18†	18 (43%)‡	6†	6 (15%)‡
Solicited systemic adverse events												
Total	1	1 (10%)	1	1 (10%)	44*	27 (54%)‡	23‡	14 (28%)‡	45*	28 (47%)‡	24*	15 (25%)‡
Post dose #1	1	1 (10%)	0	0 (0%)	12	10 (20%)	10	9 (18%)	13	11 (18%)	10	9 (15%)
Post dose #2	0	0 (0%)	1	1 (10%)	11	9 (18%)	6	4 (9%)	11	9 (15%)	7	5 (9%)
Post dose #3	N/A	N/A	N/A	N/A	13†	10 (22%)	3†	3 (7%)	13†	10 (22%)	3†	3 (7%)
Post dose #4	N/A	N/A	N/A	N/A	8	8 (19%)	4	4 (10%)	8	8 (19%)	4	4 (10%)
Laboratory abnormalities												
Total	14†	7 (70%)	3†	2 (20%)	112	33 (66%)	83	32 (64%)	127	40 (67%)	86	34 (57%)
Post dose #1	8†	5 (50%)‡	0†	0 (0%)‡	23	19 (38%)	21	14 (28%)	31	24 (40%)	21	14 (23%)
Post dose #2	6	5 (56%)	3	2 (20%)	30	19 (38%)	23	17 (37%)	36	24 (41%)	26	19 (34%)
Post dose #3	N/A	N/A	N/A	N/A	30	20 (44%)	16	13 (31%)	30	20 (44%)	16	13 (31%)
Post dose #4	N/A	N/A	N/A	N/A	30	18 (43%)	23	16 (40%)	30	18 (43%)	23	16 (40%)
Neutropenia	9	6 (60%)	2	2 (20%)	63	25 (50%)	40	20 (40%)	72*	31 (52%)	42*	22 (37%)
Grade 1	7‡	5 (50%)‡	0‡	0‡	40	24 (48%)	34	20 (40%)	47	29 (48%)	34	20 (33%)
Grade 2	2	2 (20%)	2	2 (20%)	23*	13 (26%)	6*	5 (10%)	25	15 (25%)	8	7 (12%)

(Table 2 continues on next page)

	Pilot safety cohort				Main cohort				Total			
	Pfs25H, 16 µg		Comparator vaccine		Pfs25H, 47 µg		Comparator vaccine		Pfs25H		Comparator vaccine	
	Total number	Number of individuals (%)	Total number	Number of individuals (%)	Total number	Number of individuals (%)	Total number	Number of individuals (%)	Total number	Number of individuals (%)	Total number	Number of individuals (%)
(Continued from previous page)												
Malaria												
Symptomatic malaria	11	6 (60%)	9	6 (60%)	116†	44 (88%)	84†	40 (80%)	127†	50 (83%)	93†	46 (77%)
After first dose	1	1 (10%)	1	1 (10%)	11	11 (22%)	5	5 (10%)	12	12 (20%)	6	6 (10%)
After second dose	10	6 (67%)	8	6 (60%)	18	16 (32%)	13	13 (28%)	28	22 (37%)	21	19 (34%)
After third dose	N/A	N/A	N/A	N/A	51	34 (76%)	36	26 (62%)	51	34 (76%)	36	26 (62%)
After fourth dose	N/A	N/A	N/A	N/A	36	32 (76%)	30	27 (68%)	36	32 (76%)	30	27 (68%)
Blood smear positive	19	9 (90%)	30	7 (70%)	308	48 (96%)	254	42 (84%)	327	57 (95%)‡	284	49 (82%)‡
After first dose	2	1 (10%)	2	1 (10%)	18	16 (32%)	10	9 (18%)	20	17 (28%)	12	10 (17%)
After second dose	17	8 (89%)	28	6 (60%)	45	30 (60%)	40	27 (59%)	62	38 (64%)	68	33 (59%)
After third dose	N/A	N/A	N/A	N/A	152	43 (96%)‡	126	33 (79%)‡	152	43 (96%)‡	126	33 (79%)‡
After fourth dose	N/A	N/A	N/A	N/A	93	41 (98%)‡	78	32 (80%)‡	93	41 (98%)‡	78	32 (80%)‡
Gametocyte positive	2	2 (20%)	9	5 (50%)	51	28 (56%)	48	19 (38%)	53	30 (50%)	57	24 (40%)
After first dose	0	0 (0%)	0	0 (0%)	3	3 (6%)	6	6 (12%)	3	3 (5%)	6	6 (10%)
After second dose	2	2 (22%)	9	5 (50%)	6	6 (12%)	3	3 (7%)	8	8 (14%)	12	8 (14%)
After third dose	N/A	N/A	N/A	N/A	27	20 (44%)	23	11 (26%)	27	20 (44%)	23	11 (26%)
After fourth dose	N/A	N/A	N/A	N/A	15	10 (24%)	16	10 (25%)	15	10 (24%)	16	10 (25%)

Participants were actively monitored on days 1, 3, 7, 14, and 28 after vaccination and then monthly during the long-term safety follow-up until unblinding. Medically qualified study personnel were available at all times for unscheduled visits. Solicited adverse events include local adverse events that were recorded for 14 days and systemic adverse events that were recorded for 28 days after each vaccination. Unsolicited adverse events (including symptomatic malaria), serious adverse events, and new onset of chronic illness were recorded throughout the study. Protocol-specified laboratory assessments were completed before vaccination and on days 3 and 14 after vaccination and reported in this table if observed within 28 days after vaccination. Blood smears were prepared before each vaccination, at least monthly after vaccination, or when clinically indicated. Starting 2 weeks after third and fourth vaccination, blood smears were prepared at every DSF visit. Symptomatic malaria was defined as asexual parasitaemia with axillary temperature of at least 37·5°C, clinical signs and symptoms of malaria, or both. Blood smear positive defined as at least one *Plasmodium falciparum* asexual parasite seen on blood smear. Gametocyte positive was defined as at least one gametocyte seen by one of two readers on blood smear. N/A=not applicable. *Significant by Wilcoxon rank sum for both count and rate (p≤0·05). †Significant by Wilcoxon rank sum only for count for each individual (p≤0·05); counts between vaccinations done only by count Wilcoxon rank sum test. ‡Significant by Fishers exact (p≤0·05). DSF=direct skin feeding.

Table 2: **Frequency of adverse events and serious adverse events after vaccination, by vaccine dose, cohort, and relatedness**

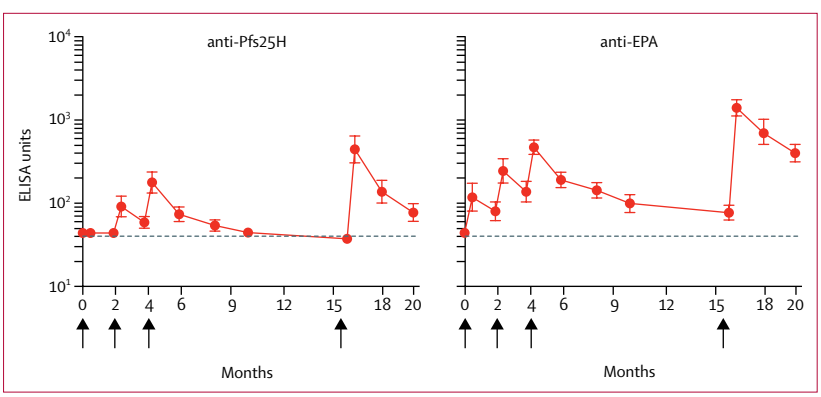


Figure 2: **Anti-Pfs25H and anti-EPA IgG ELISA titres in Mali**
Arrows indicate the day of vaccination. Only data from main cohort who received 47 µg Pfs25H-EPA/Alhydrogel vaccination presented. Note all comparator participants were below the level of detection for anti-Pfs25 responses at all timepoints. Closed circles represent geometric mean antibody titres and black bars show 95% CI. Non-responders are defined as those participants who did not have an ELISA unit of more than 44. EPA=ExoProtein A.

review by the data and safety monitoring board and sponsor. Upon unmasking, neither the number of unique participants reporting neutropenia nor the severity of neutropenia differed between the Pfs25H vaccine and comparator vaccine groups. However, in the main cohort, the overall count and rate of neutropenia (including grade 2 neutropenia) were higher in Pfs25H vaccinees than in comparator vaccinees (table 2; appendix pp 26–27). Further safety analyses are provided in the appendix (pp 19–27).

A significantly larger number of symptomatic malaria episodes occurred in the Pfs25H vaccine group than in the comparator group over the entire study period (p=0·036 by Wilcoxon rank sum for count; table 2), but this difference was not statistically significant after accounting for time at risk (p=0·082 by Wilcoxon rank sum for rate). The number of symptomatic malaria episodes in the Pfs25H vaccine group and comparator vaccine group also did not differ after any one vaccination, with increasing vaccine dose, or on a unique individual basis (table 2). Similarly, Pfs25H vaccinees and comparator vaccinees did not differ in number or incidence of blood smear-positive or gametocyte-positive events, except after the third and fourth vaccine doses, when the number of unique participants with at least one positive blood smear was higher in the Pfs25H 47 µg vaccine group than in the comparator group (table 2; appendix p 28).

No Pfs25H antibody titres were detected in Pfs25H vaccinees before vaccination or in the comparator groups

	anti-Pfs25 ELISA responses			anti-EPA ELISA responses		
	Vaccine dose 2	Vaccine dose 3	Vaccine dose 4	Vaccine dose 2	Vaccine dose 3	Vaccine dose 4
Geometric mean titre (95% CI)	93·1 (70–122)	169·6 (127–225)	422·3 (290–615)	237·4 (171–329)	468·1 (383–572)	1406·0 (1124–1759)
Non-responders*	18/45 (40%)	5/44 (11%)	1/41 (2%)	3/45 (7%)	1/44 (2%)	0/41
Geometric mean titre of responders	153·3	201·6	476·8	267·8	494·6	1406·0

Data are mean (95% CI), n/N (%), or mean. EPA=ExoProtein A.

Table 3: **Anti-Pfs25 and anti-EPA IgG ELISA titres in Mali**

at any timepoint (figure 2; table 3). Five of nine participants in the safety pilot cohort who received two doses of Pfs25H 16 µg developed a detectable response (appendix p 29). In the main cohort, antibody titres to Pfs25H were detected in only one (2%) of 50 participants after the first dose, in 27 (60%) of 45 participants after the second dose, and in 39 (89%) of 44 participants after the third dose (figure 2). Titres decreased to undetectable levels in all vaccinees by the fourth dose (1 year after the third dose). However, a clear anamnestic response developed after the fourth dose, when highest titres were achieved (geometric mean 422·3 ELISA units, 95% CI 290–615), which correlated with titres after the third dose (appendix p 33). Pfs25H titres were detected in 40 (98%) of 41 participants in the main cohort after the fourth dose. Antibody titres against EPA were detected after the first dose in 29 (59%) of 49 participants and significantly increased with each dose (figure 2), as seen previously in vaccinees in an American study.[14] Peak geometric mean antibody titres according to treatment doses at all evaluable timepoints are shown in the appendix (pp 33–32). Anti-Pfs25H titres were positively associated with anti-EPA titres at all timepoints but decreased more rapidly than did anti-EPA titres after the fourth dose (half-life of 42 days for anti-Pfs25H and 59 days for anti-EPA), which had also been reported in a previous study.[14] The median ratio at day 600 per peak was 0·19 for Pfs25H antibody titres and 0·29 for EPA antibody titres (p=0·009 by paired Wilcoxon's test).

Functional activity measured by SMFA, which includes transmission-reducing activity (ie, reduction in the

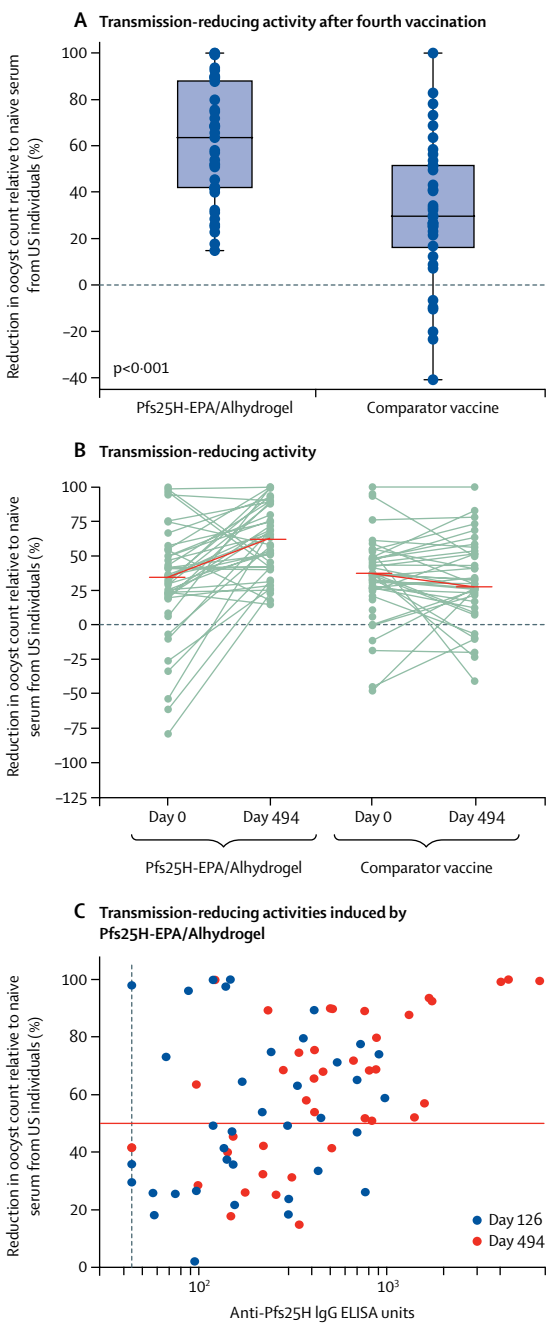


Figure 3: **Serum transmission-reducing activity after the fourth dose of Pfs25H-EPA/Alhydrogel**

(A) SMFA with sera from 14 days after the fourth dose. (B) Change in serum functional activity from day 0 to 14 days after fourth dose in individuals who received Pfs25H-EPA/Alhydrogel or comparator vaccines. Each data point is the SMFA results for an individual participant, with the black line (A) and red line (B) indicating the median. The comparator vaccines were Euvax B for the first, second, and third vaccinations and Menactra for the fourth vaccination. (C) Non-linear association of antibody titers with serum activity (p value 0·01 GEE) gives confirmation in participants with the highest anti-Pfs25 ELISA titres and corresponding high functional activity (by SMFA) that the functional responses are Pfs25-dependent. The red line indicates the threshold representing significant biological activity (50% transmission-reducing activity), and the dotted vertical grey line indicates 44 ELISA units as the limit of detection of the assay. SMFA=standard membrane feeding assay. GEE=generalised estimating equations. Day 126 is 14 days after dose 3; day 494 is 14 days after dose 4.

number of oocysts per mosquito) and transmission-blocking activity (ie, reduction in the proportion of mosquitoes infected), was assessed on sera from all available participants in the main cohort at baseline and 14 days after the third and fourth vaccination. A few participants had significant transmission-reducing activing at baseline (appendix pp 30–32). Average transmission-reducing activing by SMFA was not significantly different in Pfs25H vaccinees versus comparator vaccinees after the third dose (41% of Pfs25H vaccinees *vs* 28% of comparator vaccinees; p=0·09 by Wilcoxon test), but transmission-reducing activity was significantly higher in the vaccinated group than in the comparator group after the fourth dose (p<0·001; figure 3A–B; appendix pp 29–32). Anti-Pfs25H titres generally correlated with functional activity (figure 3C). Transmission-blocking activity did not differ between Pfs25H vaccinees and comparator vaccinees (figure 4A), but SMFA used for this purpose is known to give highly variable results that depend on infection intensity in control mosquitoes.[19,20]

To confirm that functional activity was mediated by vaccine-specific antibodies, SMFA was done using IgG purified from five selected individuals with high titres (>1000 units) and high transmission-blocking activity (figure 5). For two individuals with highest titres and sufficient yield of purified IgG, Pfs25H-specific IgG was then depleted on affinity columns, which resulted in substantial or complete loss of activity measured in SMFA (participants 1 and 2). Thus, functional activity in vaccinees was contained in the IgG fraction directed against Pfs25H. To determine whether increased activity after the fourth dose was associated with quality of antibody, antibody avidity to Pfs25H was measured in a modified ELISA using a chaotropic agent (figure 6). Using a mixed-effects statistical model of the log of the avidity index, relative avidity to Pfs25H differed significantly between day 70 (14 days after the second dose) and day 126 (14 days after the third dose; p=0·026) as well as between day 70 and day 494 (14 days after the fourth dose; p<0·001; appendix pp 34–35). Increased activity was therefore associated with increased antibody avidity.

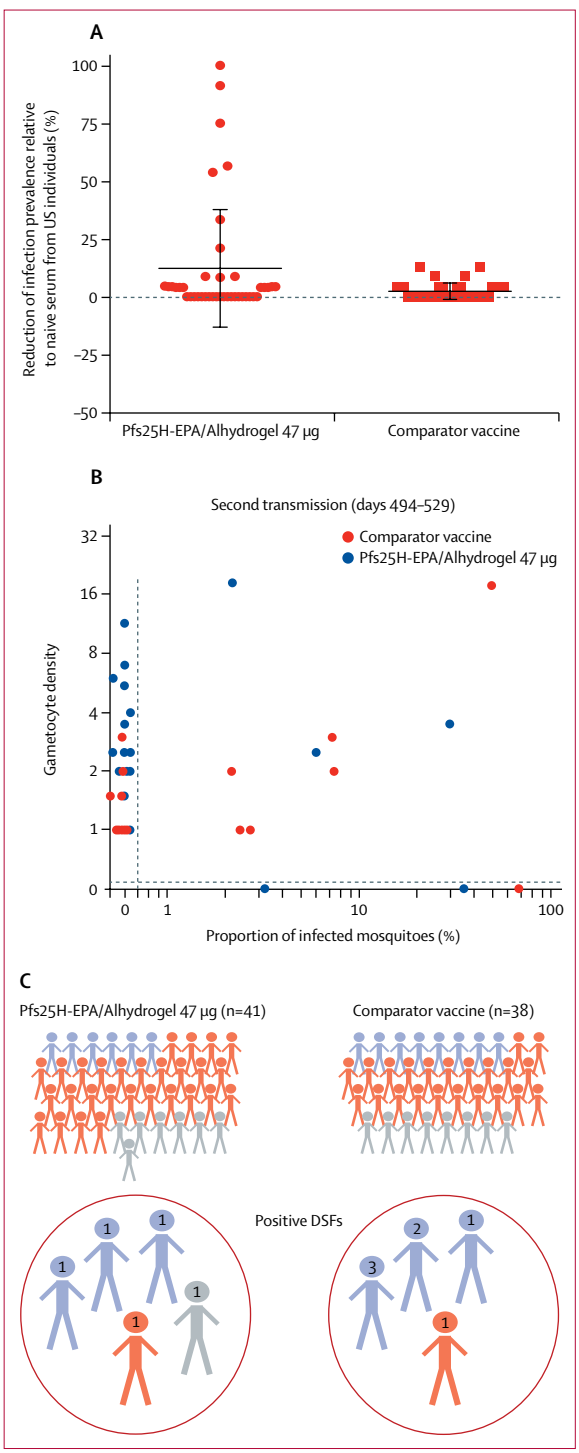


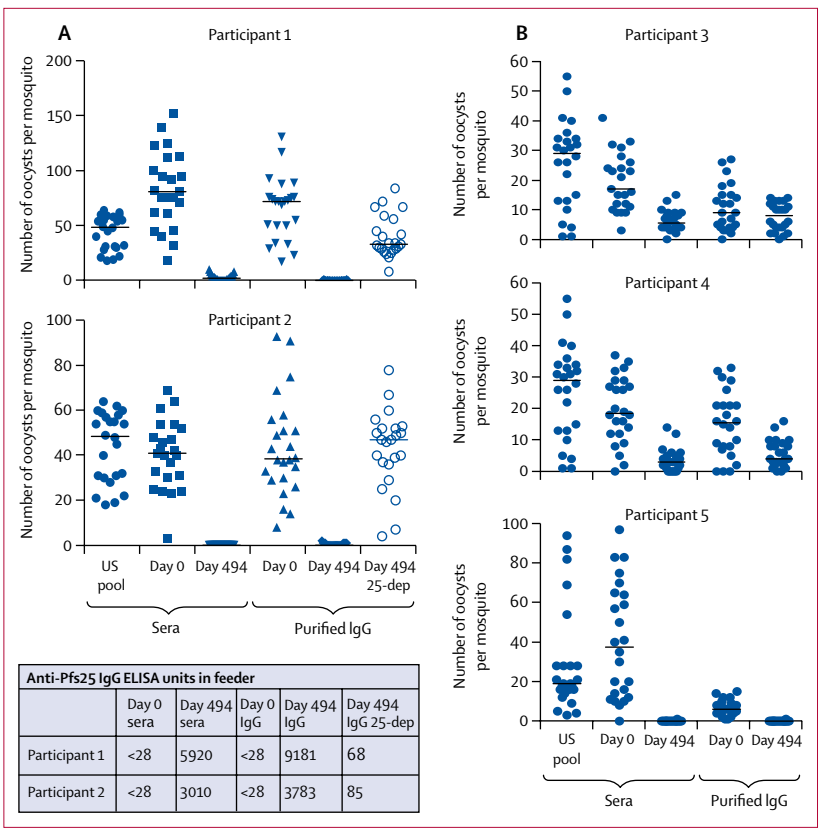
Figure 4: **Serum transmission-blocking activity and DSF summary after the fourth dose of Pfs25H-EPA/Alhydrogel**

(A) A subset of participants who received Pfs25H-EPA/Alhydrogel, but none who received comparator vaccine, had activity that reduced infection prevalence by more than 20% 14 days after the fourth vaccine dose (day 494). (B) The relationship between gametocyte density (gametocytes per 1000 white blood cells) in the study participants and proportion of infected mosquitoes (number of oocyst infected mosquitoes/number of dissected mosquitoes × 100) resulting from DSF assays each week for 6 weeks after the fourth vaccine dose in the second season, stratified by test and comparator vaccine groups. Most but not all DSF with at least one oocyst-infected mosquito resulted from assays on study participants with detectable gametocytes, and conversely, many study participants with detectable gametocytemia did not transmit parasites to mosquitoes (19 events for Pfs25H *vs* 11 events for comparator vaccine). DSF where values for both gametocyte density and proportion mosquitoes infected were zero (214 for Pfs25H *vs* 205 for comparator vaccine) are not displayed. Zero values are set to 0·5 for display on the log scale plot, as demarcated by the dashed line. (C) A small number of subjects who received vaccine (N=5) or comparator (N=4) transmitted parasites to colony-raised mosquitoes at one or more timepoints. Each study participant is also presented as having had any gametocytaemia (blue), any parasitaemia (orange), or no parasitaemia (gray) throughout the DSF period. The numeric value is number of positive DSF during follow-up. DSF=direct skin feed. SMFA=standard membrane feeding assay.

Using a linear GEE model for the log of the oocyst count ratio from the SMFAs, and using the square root of the ELISA values on sera from 14 days after the third and fourth doses, we found that anti-Pfs25H concentrations were associated with SMFA values (p=0·01; figure 3C). Using this same model, which was previously published for EC_{50} calculations, but using day 0 transmission-reducing activity as the control (given pre-existing activity in Malian adults), the estimated EC_{50} of serum anti-Pfs25H concentration is 49 µg/mL (95% CI 22·5–78·2; by GEE method), which is similar to the concentration seen previously in the US phase 1 study (EC_{50} 57·2 µg/ml, 95% CI 44·7–76·8).[14] Using their day 0 transmission-reducing activity as control, the mean transmission-reducing activity at day 126 for Pfs25H vaccinees was 2% (T-based 95% CI –13 to 16); at day 494, the mean transmission-reducing activity was 27% (6–47); and at days 126 and 494 combined, the mean transmission-reducing activity was 14% (robust 95% CI 0·73–26·32).

627 DSF assays were completed during the entire study. After the third vaccination, DSF assays were piloted in parasite carriers only and at various timepoints. Starting 14 days after the fourth dose (booster dose), 79 participants (41 Pfs25H vaccinees and 38 comparator vaccinees) completed 461 DSF assays (figure 1); two comparator vaccinees did not complete a DSF assay because of an unrelated adverse event (trauma) and consent withdrawal, and one Pfs25H vaccinee withdrew consent before the DSF. Of the DSF assays done after the fourth vaccination, 12 (3%) were positive (five Pfs25H vaccines and seven comparator vaccinees; figure 4B). Nine (11%) individuals (five Pfs25H vaccinees and four comparator vaccinees) had positive DSF results (ie, had infected at least one mosquito; figure 4C; table 4).

Of the 14 individuals who were gametocytaemic at the time of a DSF assay, six (43%) participants (three Pfs25H vaccinees and three comparator vaccinees) infected at least one mosquito in their concurrent DSF assay. A conditional exact test for the proportion of participants with a positive DSF assay result among those with concurrent detectable gametocytaemia found no significant difference between Pfs25H and comparator vaccine groups (p=1). We also found no significant difference in the proportions of infective participants in the Pfs25H and comparator vaccine groups when comparing DSF results with concurrent detectable gametocytes (p=1 by conditional exact test). Of the many other statistical methods and outcomes investigated on the basis of DSF assays results, no significant differences were found between the Pfs25H and comparator vaccine groups in average oocyst count, the number of mosquito infections, number of positive DSF assay results, or number of participants infecting at least one mosquito (figure 4B; table 4).



Anti-Pfs25 IgG ELISA units in feeder					
	Day 0 sera	Day 494 sera	Day 0 IgG	Day 494 IgG	Day 494 IgG 25-dep
Participant 1	<28	5920	<28	9181	68
Participant 2	<28	3010	<28	3783	85

Figure 5: **Functional activity mediated by vaccine-specific antibodies**
(A–B) SMFA of sera and IgG after the fourth vaccine dose from five individuals who received Pfs25H-EPA/Alhydrogel 47 µg. (A) Two individuals with relatively high antibody titres had high functional activity in purified IgG fraction ablated by depletion of Pfs25-specific IgG on an antigen-affinity column. 25-dep=depletion of Pfs25-specific IgG. SMFA=standard membrane feeding assay. EPA=ExoProtein A.

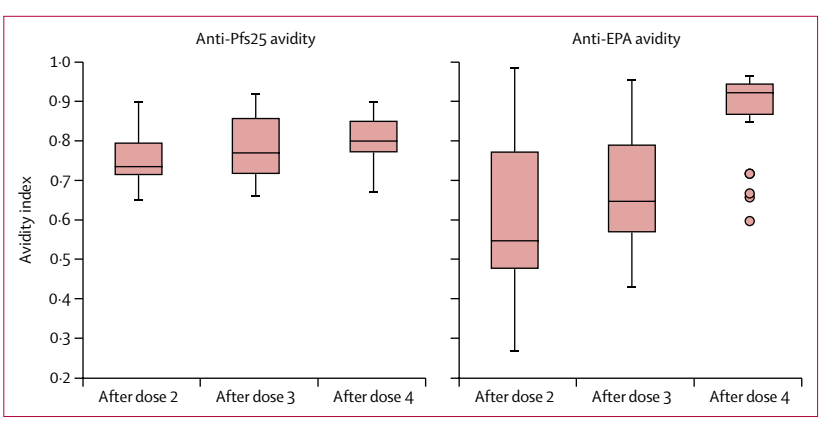


Figure 6: **Avidity index in sera after subsequent vaccinations**
Avidity testing was done on sera taken 14 days after the second vaccine dose (study day 70), 14 days after the third dose (study day 126), and 14 days after the fourth dose (study day 494). EPA=ExoProtein A.

Discussion

Transmission-blocking vaccines have been envisioned since the 1970s as an interventional concept to support malaria elimination efforts. A parasite's transmission to a mosquito represents a parasite bottleneck, with usually less than five oocysts per mosquito, whereas an infected human can harbour as many as 10×10^{13} asexual stage parasites in the blood stream. However, earlier attempts to develop a transmission-blocking vaccine foundered on

	Number of DSF assays completed	Number of positive DSF results	Number of positive DSF results with gametocytaemic participants	Average mosquito feeding frequency	Average mosquito survival frequency	Number of infected mosquitoes	Median number of infected mosquitoes	Median number of oocysts per infected mosquito
Pfs25H-EPA/Alhydrogel 47 µg	238	5 (2%)	3 (60%)	93%	70%	31 (<1%)	1 (1–14)	1 (1–7)
Comparator vaccine	223	7 (3%)	6 (86%)	93%	71%	51 (1%)	3 (1–23)	2 (1–7)
Total	461	12 (3%)	9 (75%)	93%	71%	82 (1%)	2 (1–23)	2 (1–7)

Data are n, n (%), %, or median (range). DFS=direct skin feeding.

Table 4: **DSF assay outcomes by group**

poor immunogenicity[9,10] or reactogenicity related to the adjuvant-formulated products.[11] We recently showed that chemical conjugation to the carrier protein EPA yields a Pfs25 particle immunogen[21] that was safe and immunogenic when formulated with the aluminum adjuvant Alhydrogel and administered to malaria-naive US volunteers.[14] We report here the first trial results of a transmission-blocking vaccine in the field, which show that the recombinant protein–protein conjugate vaccine Pfs25H-EPA/Alhydrogel is safe and induces functional serum activity in Malian adults similar to the activity induced in US adults.[14]

Although the mean antibody titre in Malians was about half that of US vaccinees,[14] the pattern of the response was similar between the two studies. Malians showed no pre-existing responses to Pfs25H and generally no responses after the first dose, which is consistent with post-fertilisation expression in the mosquito.[22] Antibody titres increased progressively with each dose and in response to the final boost 1 year later. The reason for the modestly lower antibody titres in Malians is unknown to us. Individuals with HIV or viral hepatitis infections were excluded. Responses to other vaccines are also lower in resource-poor tropical communities than in developed nations, and malnutrition and co-infections, including parasitic infections, can suppress vaccine responses.[23] Of note, our trial cohort was largely healthy young adults, and helminth infections were uncommon, which is presumably a beneficial result of the periodic mass de-worming campaigns. Malaria itself can suppress vaccine responses,[24] and we are now assessing anti-malarial treatment before vaccinations in Mali.

Despite modestly lower antibody titres in Malian vaccinees than in US vaccinees, post-vaccination serum functional activity was similar. In both trials, 27% of vaccinees attained a level of serum functional activity after the fourth vaccination that reduced oocyst burden in mosquitoes by more than 80% (as assessed by SMFA). The relationship between SMFA and in-vivo measurements of transmission-blocking activity has not been studied in humans. Modelling data suggest that serum reduction of oocyst numbers by SMFA (ie, transmission-reducing activity) correlates with a reduced prevalence of infected mosquitoes (ie, transmission-blocking activity) when oocyst numbers are low in the control mosquitoes receiving non-immune serum.[25] However, oocyst counts are typically low (<5 oocysts per midgut) in wild-caught mosquitoes.[26] Notably, serum activity in US vaccinees was largely lost by 8 weeks after the fourth dose when Pfs25H titres had decreased by more than half.[14] We therefore expected a similar decrease in activity with titres seen in this study.

Malian adults had pre-existing functional activity that reduced the parasite transmission that was measured in SMFA to variable degrees. Some individuals had high activity that persisted over time. Naturally acquired activity has been associated with antibody against known pre-fertilisation malaria antigens such as Pfs230.[26] Although this pre-existing activity probably persisted in vaccinees throughout the trial (as it did in comparators), we did not find a strong correlation between functional activity measured at baseline and after the fourth vaccination, whereas Pfs25H titres were highly correlated with activity. We also showed that high serum activity in Pfs25H vaccinees was mediated by Pfs25-specific antibodies. Thus, we concluded that the functional serum activity measured after the fourth dose in most vaccinees was largely related to immunisation.

Pfs25H-EPA/Alhydrogel has significant limitations. Four vaccine doses were needed to achieve clinically significant serum functional activity, both in the USA[14] and in Mali. Regimens that can be used with less than four doses will be easier to implement, and we are exploring additional platforms such as potent adjuvants for dose-sparing benefits. Furthermore, Pfs25H titres decreased rapidly after each post-dose peak, and the serum functional activity at 2 weeks after the fourth dose had disappeared within 8 weeks in the US trial.[14] Pfs25 is a post-fertilisation antigen, and therefore vaccine responses are not boosted during naturally occurring infections. The short period of activity is a serious limitation for any vaccine, and for practical purposes the activity of transmission-blocking vaccines should persist for at least a season of transmission. In mice, we found that potent adjuvants, such as the liposomal adjuvant ALF-Q that incorporates QS21 and the TLR4 ligand GLA, can induce more durable antibody responses to Pfs25H vaccines,[27] and we are now evaluating similar adjuvant in humans.

Finally, we have also developed a vaccine based on the pre-fertilisation antigen Pfs230 to study either alone or in combination with Pfs25H-EPA in human trials. Some naturally exposed individuals acquire antibodies against Pfs230 that correlate with serum functional activity,[26] suggesting that vaccine responses might be boosted during infection. Complement enhances Pfs230 antibody activity,[28] and this effect might reduce the antibody titre necessary to block transmission. We also hypothesise that the combination of activities against pre-fertilisation and post-fertilisation antigens will exceed their individual activities, although evidence for this has not been conclusive in preclinical studies.

As transmission-blocking vaccines advance in the field, new approaches to measure efficacy will be needed.[29] A definitive efficacy trial for reducing malaria incidence might need several communities and hence be relatively large and expensive.[30] As an interim endpoint, we explored DSF assays to measure vaccine activity, using colony-raised mosquitoes that fed directly on participants.[31] Weekly DSF was safe and well tolerated and yielded infected mosquitoes, although the rate of mosquito infection was low (roughly 0·3%). We are now expanding our DSF capacity to achieve sample sizes needed to confirm vaccine activity in future trials in Mali.

In conclusion, the Pfs25H-EPA/Alhydrogel vaccine candidate was safe and immunogenic in Malian adults and induced significant serum activity after four doses that reduced parasite transmission to mosquitoes in a laboratory assay. In this first field trial of a transmission-blocking vaccine against malaria, functional immunogenicity in the target population was achieved despite lifetime exposure to *P falciparum*; however, transmission-blocking activity was incomplete, and Pfs25H antibody titres decreased rapidly. Future studies should seek to increase and prolong functional antibody activity, possibly by combining Pfs25 with another antigen such as Pfs230, and to measure vaccine activity against naturally circulating infections.

Contributors

IS and SAH were the principal investigators. IS, SAH, EEG, MPF, YW, MBC, SFT, OKD, and PED designed the trial with contributions from all authors in the review of the approved final version and additional amendments. IS, MHA, MK, KS, IT, MAG, MD, IB, and MBC collected the data. MAG, SS, MD, IB, MBC, OM, and CA completed the study laboratory endpoints. KR, DLN, DLJ, NJM, DZ, and PED developed the vaccine. RM developed the database for the study. AD and KN prepared the vaccines for injection. EEG, MPF, and CA completed the statistical analysis. IS, SAH, EEG, MBC, CA, YW, OKD, and PED interpreted data and results.

Declaration of interests

We declare no competing interests.

References

1 GBD 2015 Mortality and Causes of Death Collaborators. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* 2016; **388:** 1459–544.

2 Dowdle WR. The principles of disease elimination and eradication. *Bull World Health Organ* 1998; **76:** 22–25.

3 Carter R, Chen DH. Malaria transmission blocked by immunisation with gametes of the malaria parasite. *Nature* 1976; **263:** 57–60.

4 Gwadz RW. Successful immunization against the sexual stages of *Plasmodium gallinaceum*. *Science* 1976; **193:** 1150–51.

5 Carter R, Kaushal DC. Characterization of antigens on mosquito midgut stages of *Plasmodium gallinaceum*. III. Changes in zygote surface proteins during transformation to mature ookinete. *Mol Biochem Parasitol* 1984; **13:** 235–41.

6 Grotendorst CA, Kumar N, Carter R, Kaushal DC. A surface protein expressed during the transformation of zygotes of *Plasmodium gallinaceum* is a target of transmission-blocking antibodies. *Infect Immun* 1984; **45:** 775–77.

7 Duffy PE, Pimenta P, Kaslow DC. Pgs28 belongs to a family of epidermal growth factor-like antigens that are targets of malaria transmission-blocking antibodies. *J Exp Med* 1993; **177:** 505–10.

8 Barr PJ, Green KM, Gibson HL, Bathurst IC, Quakyi IA, Kaslow DC. Recombinant Pfs25 protein of *Plasmodium falciparum* elicits malaria transmission-blocking immunity in experimental animals. *J Exp Med* 1991; **174:** 1203–08.

9 Kaslow DC. Transmission-blocking vaccines: uses and current status of development. *Int J Parasitol* 1997; **27:** 183–89.

10 Ockenhouse CF, Sun PF, Lanar DE, et al. Phase I/IIa safety, immunogenicity, and efficacy trial of NYVAC-Pf7, a pox-vectored, multiantigen, multistage vaccine candidate for *Plasmodium falciparum* malaria. *J Infect Dis* 1998; **177:** 1664–73.

11 Wu Y, Ellis RD, Shaffer D, et al. Phase 1 trial of malaria transmission blocking vaccine candidates Pfs25 and Pvs25 formulated with montanide ISA 51. *PLoS One* 2008; **3:** e2636.

12 Qian F, Wu Y, Muratova O, et al. Conjugating recombinant proteins to *Pseudomonas aeruginosa* ExoProtein A: a strategy for enhancing immunogenicity of malaria vaccine candidates. *Vaccine* 2007; **25:** 3923–33.

13 Wu Y, Przysiecki C, Flanagan E, et al. Sustained high-titer antibody responses induced by conjugating a malarial vaccine candidate to outer-membrane protein complex. *Proc Natl Acad Sci USA* 2006; **103:** 18243–48.

14 Talaat KR, Ellis RD, Hurd J, et al. Safety and Immunogenicity of Pfs25-EPA/Alhydrogel(R), a transmission blocking vaccine against *Plasmodium falciparum*: an open label study in malaria naive adults. *PLoS One* 2016; **11:** e0163144.

15 Food and Drug Administration Center for Biologics Evaluation and Research. Guidance for industry. Toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials. Washington, DC: US Department of Health and Human Services, Food and Drug Administration Center for Biologics Evaluation and Research, 2007.

16 Carey V. Generalized estimation equation solver. 2015. https://cran.r-project.org/web/packages/gee/gee.pdf (accessed Aug 1, 2017).

17 Bates D, Machler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. *J Stat Softw* 2014; published online Oct 7. DOI:10.18637/jss.v067.i01.

18 Kuznetsova A, Brockhoff P, Christensen R. Tests in linear mixed effects models. 2016. https://cran.opencpu.org/web/packages/lmerTest/lmerTest.pdf (accessed Aug 1, 2017).

19 Miura K, Deng B, Tullo G, et al. Qualification of standard membrane-feeding assay with *Plasmodium falciparum* malaria and potential improvements for future assays. *PLoS One* 2013; **8:** e57909.

20 Churcher TS, Blagborough AM, Delves M, et al. Measuring the blockade of malaria transmission—an analysis of the standard membrane feeding assay. *Int J Parasitol* 2012; **42:** 1037–44.

21 Shimp RL Jr, Rowe C, Reiter K, et al. Development of a Pfs25-EPA malaria transmission blocking vaccine as a chemically conjugated nanoparticle. *Vaccine* 2013; **31:** 2954–62.

22 Vermeulen AN, Ponnudurai T, Beckers PJ, Verhave JP, Smits MA, Meuwissen JH. Sequential expression of antigens on sexual stages of *Plasmodium falciparum* accessible to transmission-blocking antibodies in the mosquito. *J Exp Med* 1985; **162:** 1460–76.

23 Labeaud AD, Malhotra I, King MJ, King CL, King CH. Do antenatal parasite infections devalue childhood vaccination? *PLoS Negl Trop Dis* 2009; **3:** e442.

24 Cunnington AJ, Riley EM. Suppression of vaccine responses by malaria: insignificant or overlooked? *Expert Rev Vaccines* 2010; **9:** 409–29.

25 Miura K, Swihart BJ, Deng B, et al. Transmission-blocking activity is determined by transmission-reducing activity and number of control oocysts in *Plasmodium falciparum* standard membrane-feeding assay. *Vaccine* 2016; **34:** 4145–51.

26 Graves PM, Carter R, Burkot TR, Quakyi IA, Kumar N. Antibodies to *Plasmodium falciparum* gamete surface antigens in Papua New Guinea sera. *Parasite Immunol* 1988; **10:** 209–18.

27 Radtke AJ, Anderson CF, Riteau N, et al. Adjuvant and carrier protein-dependent T-cell priming promotes a robust antibody response against the *Plasmodium falciparum* Pfs25 vaccine candidate. *Sci Rep* 2017; **7:** 40312.

28 Quakyi IA, Carter R, Rener J, Kumar N, Good MF, Miller LH. The 230-kDa gamete surface protein of *Plasmodium falciparum* is also a target for transmission-blocking antibodies. *J Immunol* 1987; **139:** 4213–17.

29 Delrieu I, Leboulleux D, Ivinson K, Gessner BD, Malaria Transmission Blocking Vaccine Technical Consultation Group. Design of a phase III cluster randomized trial to assess the efficacy and safety of a malaria transmission blocking vaccine. *Vaccine* 2015; **33:** 1518–26.

30 White MT, Verity R, Churcher TS, Ghani AC. Vaccine approaches to malaria control and elimination: Insights from mathematical models. *Vaccine* 2015; **33:** 7544–50.

31 Brickley EB, Coulibaly M, Gabriel EE, et al. Utilizing direct skin feeding assays for development of vaccines that interrupt malaria transmission: a systematic review of methods and case study. *Vaccine* 2016; **34:** 5863–70.